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# Pulmonary Infections Due to *Legionella pneumophila* in Immunocompromised Patients

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**Abstract:** The numbers of immunocompromised individuals increase as programmes for organ transplantation and chemotherapy of malignant disease advance. Pulmonary infections, which progress rapidly, are one of the major clinical problems in these groups. This study was designed to evaluate the microbiology of pneumonia in immunocompromised patients and to determine the role of *Legionella pneumophila.*

Immunocompromised patients receiving antineoplastic chemotherapy, diagnosed as pneumonia with clinical and radiographic findings and routine laboratory tests were included in the study. For microbiological diagnosis, expectorated sputum was cultured for *L. pneumophila.* In addition to standard bacterial, fungal and mycobacterial media, specimens were examined microscopically. Direct flourescent antibody (DFA) assay was used for antigen detection of *L. pneumophila.* By indirect flourescent antibody (IFA) assay the sera of the patients were screened for *L. pneumophila.*

Of 74 sputum samples, 7 (9.5%) specimens yielded *L. pneumophila.* The other isolates were *Escherichia coli (n:3),* Enterobacter sp. *(n:2),* Aspergillus sp. *(n:3), Mycobacterium tuberculosis (n:3), Staphylococcus aureus (n:1),* yeasts *(n:10),* Nocardia sp. *(n:2)* and flora members *(n:43).* Antibiotic supplemented media and acid treatment was found superior for the isolation of *L. pneumophila.* Direct microscopic evaluation was necessary to support the culture results. DFA assay had 85% sensitivity and 100% specificity. Of 3 sera screened by IFA for *L. pneumophila* antibodies, 2 had the titres 1/256, indicating the disease.

Our results showed that *L. pneumophila* has an important role among the respiratory pathogens in immunocompromised patients, and it may be useful if techniques for isolation and identification of this pathogen are added to routine culturing programmes.

**Key Words:** *L. pneumophila,* immunocompromised patients, pulmonary infection, diagnosis.

## Introduction

During the past decade the number of immunocompromised patients has increased in parallel to the increased use of high-dose chemotherapy and immunosuppressive drugs. A compromised host who has one or more defects in the body's natural defence mechanisms, is predisposed to severe, often life-threatening infections (1,2).

A sensible approach to the diagnosis and treatment of infections in these patients is to begin with an appreciation of the predisposing factors, because each is associated with an array of pathogens. The isolation and identification of a specific pathogen permits appropiate antibiotic management of patients with pulmonary infections (1,2).

This study was designed to evaluate the microbiology of pneumonia in immunocompromised patients at the Osmangazi University Medical Faculty Hospital and to compare the results with those of previous reports. The role of *L. pneumophila* and microbiological techniques for isolation and identification of *L. pneumophila* were also discussed.

## Materials and Methods

Immunocompromised patients with pneumonia admitted to the Department of Chest Disease and Haematology Clinics of the Medical Faculty of Osmangazi University over a 1- yr period from 1 November 1995 to 31 March 1997 were eligible for the study. Criteria for hospitalization were at the discretion of the clinic from

which the patient was admitted. Outpatient diagnostic and theurapeutic interventions were performed. Inclusion criteria were as follows:

1) patients > 20 yr of age

2) pulmonary infiltrate on chest radiograph, and

3) clinical findings of one "major criterium": cough, sputum production of fever > 37.8°C; or two "minor criteria": pleuritic chest pain, dyspnea, altered mental status, pulmonary consolidation by physical examination or white blood cell count > 12.000/$mm^3$. Patients were excluded if chest roentgenograms did not reveal that infiltrate or radiographic abnormalities were attributable to a non-infectious aetiology. Neutropenic patients receiving antineoplastic chemotherapy were included in the study. The patients were considered neutropenic if the granulocyte count was < 1.000/$mm^3$.

Routine tests obtained upon admission included chest roentgenograms, complete blood count and serum electrolytes (3).

For microbiological diagnosis, expectorated sputum was obtained. Standard bacterial cultures were performed by inoculation of the specimen onto 5% sheep blood, chocolate, and Eosin Methylene Blue (EMB) (Difco) agars. Mycobacterial cultures (Bactec system and Löwenstein Jensen medium-Difco) and fungal cultures (Sabouraud Dextrose Agar-SDA, Difco) were also made.

The culture for Legionella sp. was made using Buffered Charcoal Yeast Extract Agar (BCYE) and BMPA containing cefamandol, polymyxin B and anisomycin (BCYE-selective agars, BCYE-s) (Oxoid). The culture results before and after acid treatment of sputum (HCl-KCl buffer, ph: 2.2) with BCYE and BCYE-selective agars were compared. All plates were incubated at 37°C for 24-28 h in an aerobic atmosphere. After examination and recording of the results, blood agar, EMB and SDA plates were discarded, whereas BCYE and BCYE-s agar plates were incubated for 5 days. All isolates were identified by standard procedures and then by the Sceptor system. Colonies suspected for Legionella were removed and stained with Gram's stain. When Gram-negative coccobacillary or filamentous forms resembling the members of the family Legionellaceae were observed, biochemical tests were performed, including catalase, oxidase, urease, nitrate, gelatinase and hippurate hydrolysis tests.

DFA for the antigen of *L. pneumophila* serogroup 1-14 was done on sputum (Fresenius Diagnostics). For *L. pneumophila* serogroup 1-8, serologic tests were done by IFA (Fresenius Diagnostics). For this purpose, a single set of sera was collected from 3 patients and screened for *L. pneumophila* serogroup 1-8 by IFA assay.

For microscopic evaluation, all sputum samples were stained with Gram's and Erhlich Ziehl Neelsen (EZN) stains. Sputum smears with a paucity of flora members and a large number of neutrophils and small, pleomorphic faintly staining Gram-negative rods supported the diagnosis if growth was obtained by culture (3,4).

A total of 74 immunocompromised patients were included in this study. The distributions according to age, sex and primary pathology are listed in Table 1.

Table 1. Characteristics of patients.

| Characteristics | | No of patients (No: 74) |
|---|---|---|
| Mean age, yr (range) | | 57.3 (22-75) |
| Male / Female | | 39 / 9 |
| Primary pathology | CLL | 7 |
| | NHL | 9 |
| | ANLL | 3 |
| | HD | 5 |
| | ALL | 1 |
| | Renal transplant | 2 |
| | Bronchial carcinoma | 44 |
| | Malignant mesothelioma | 3 |

ANLL: Acute nonlymphocytic leukemia
NHL: Non-Hodgkin lymphoma
ALL: Acute lymphoblastic leukemia
HD: Hodgkin's disease
CLL: Chronic lymphocytic leukemia

If the patients were diagnosed with pneumonia due to *L. pneumophila*, they were treated with erythromycin or roxitromycin. Improvement of the patient was defined as clinical improvement with negative microbiological diagnostic test.

## Results

Of 74 sputum specimens, 7 specimens yielded *L. pneumophila* by culturing and direct microscopic examination. The distribution of organisms isolated from sputum samples is listed in Table 2.

*L. pneumophila* was isolated from the sputum samples of 7 patients. The characteristics and outcomes of these patients are given in Table 3.

Table 2. The distribution of organisms isolated from sputum samples.

| ISOLATE | No | % |
|---|---|---|
| *L. pneumophila* | 7 | 9.5 |
| *E. coli* | 3 | 4.1 |
| Enterobacter sp. | 2 | 2.7 |
| Aspergillus sp. | 3 | 4.1 |
| *M. tuberculosis* | 3 | 4.1 |
| *S. aureus* | 1 | 1.3 |
| Yeast | 10 | 13.5 |
| Nocardia sp. | 2 | 2.7 |
| Flora members | 43 | 58.0 |

Of 7 sputum specimens including *L. pneumophila*, DFA assay yielded positive results for 6 specimens (85% sensitivity) and no cross reaction was present. Of 3 sera screened by IFA for *L. pneumophila* antibodies, the titres were 1/256 for 2 patients, indicating acute disease, and negative for one patient. DFA and antibody detection results supported the results obtained by culture and microscopic evaluation. The comparison of culturing results with DFA and serology results is shown in Table 4.

Table 3. The characteristics of the patients.

| Patient No | Age (yr) | Sex | Diagnosis | Cyctotoxic therapy and/or Corticosteroid | Symptoms | Neutropenia | Chest X-ray | Therapy | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1(SC) | 62 | M | Bronchial Cancer | Yes | Cough, malaise | No | Heterogenic density | Roxitromycin | Improved |
| 2(İY) | 65 | M | Bronchial Cancer | Yes | Dyspnea, productive cough, fever, Malaise | No | Bilateral patchy infiltrate | Roxitromycin | Imropved |
| 3(EÇ) | 51 | M | Bronchial Cancer | Yes | Bloody sputum Fever, malaise | No | Infiltration and cavitaray lesion in left lower lobe | Roxitromycin | Improved |
| 4(NY) | 70 | M | Bronchial Cancer | Yes | Dyspnea, cough, Chest pain, fever Malaise | No | Patchy density in right lower lobe, pleural effusion | (He refused therapy) | Improved |
| 5(NB) | 52 | M | SLE | Yes | Cough | Yes | Bilateral patchy infiltrate | Eryhromycin | Improved |
| 6(AP) | 69 | M | KLL | Yes | Productive cough, Fever | Yes | Bilateral patchy infiltrate | Eryhromycin | Improved |
| 7(AY) | 63 | F | Lymphoma | Yes | Non-productive cough, fever | Yes | Unilateral patchy density | Eryhromycin | Improved |

Table 4. The results obtained by culturing, DFA and IFA assays.

| Patient No | CULTURE | | | | DFA | SEROLOGY |
|---|---|---|---|---|---|---|
| | BCYE | | BCYE-S | | | |
| | Acid treatment | No acid treatment | Acid treatment | No acid treatment | | |
| 1 | Positive | Positive | Positive | Positive | Positive | NT |
| 2 | Positive | Positive | Positive | Positive | Positive | 1/256 |
| 3 | Positive | Positive | Positive | Positive | Negative | 1/256 |
| 4 | Positive | Positive | Positive | Positive | Positive | NT |
| 5 | Positive | Positive | Positive | Positive | Positive | NT |
| 6 | Negative | Negative | Positive | Negative | Positive | NT |
| 7 | Positive | Negative | Positive | Positive | Positive | Negative |

NT: Not tested

The isolation of *L. pneumophila* was found to be difficult with BCYE agar. However, BCYE-s agar allowed us to see the typical colonies easily, and pure culture was easily obtainable from this medium. In our study, all *L. pneumophila* isolates could be isolated as a pure culture by using BCYE-s agar whereas the isolates were inhibited by the flora members and not distinguished by BCYE agar.

After acid treatment of sputum samples, the flora members were inhibited, and thus isolation was easy, as BCYE-s agar. When the sputum was acid treated and inoculated onto BCYE agar, growth was obtained in 6 samples, similar to inoculating onto BCYE-s agar without acid treatment. But all samples were found positive for *L. pneumophila* when both acid treatment and inoculating onto BCYE-s agar was done. Thus, both acid treatment and using selective agar were found to be more useful for the isolation of *L. pneumophila.*

**Discussion**

In 1976, an outbreak of pneumonia occurred at a hotel at the site of the American Legion Convention in Philadelphia. The discovery of *L. pneumophila* and Legionnaires' disease was after this outbreak. *L. pneumophila* can survive in a wide range of environmental conditions. The distribution of *L. pneumophila* on man-made aquatic reservoirs, including water distribution systems and cooling towers, are related to epidemiology. The incidence of Legionnaires' disease is dependent on the degree of contamination of the organism in the aquatic reservoir and the susceptibility of the persons, availability of specialized laboratory tests and their application to the infected patients (1,5-7).

Numerous studies have shown *L. pneumophila* among the three most common microbial aetiologies of community-acquired pneumonia. It is suggested by CDC investigations that only 3% of sporadic cases of Legionnaries' disease are correctly diagnosed. The incidence of nosocomial pneumonia by Legionella sp. is dependent on the degree of colonization, the number of immunosupressed host and the availability of culture methods. Cigarette smoking, chronic lung disease, advanced age and immunosupression have been implicated as risk factors. Excess alcohol intake, renal failure and surgery are major predisposing factors, with transplant recipients at highest risk (1,7).

The aim of this study was to determine the distribution of respiratory pathogens in immunocompromised patients and the role of *L. pneumophila.* All of the patients included in this study were receiving immunosupressive therapy for the treatment of their primary pathologies.

In granulocytopenia and cellular immune dysfunction, Gram-negative bacterial infections due to the members of Enterobacteriaceae and *L. pneumophila* is common. In our study, *E. coli* was isolated from 3, Enterobacter sp. was isolated from 2 and *L. pneumophila* was isolated from 7 sputum samples. Granulocytopenic patients are also susceptible to infections caused by Gram-positive bacteria, including *S. aureus, S. epidermidis* and streptococci. Also, fungi such as candida sp. and Aspergillus sp. cause infections. In our study, Aspergillus sp. was isolated from 3 specimens, yeast from 10 specimens and *S. aureus* from 1 specimen. *M. tuberculosis* and Nocardia sp, which are also common in cellular immune dysfunction, were also isolated. This distribution of causative agents is similar to that in the literature (1-3, 8-10).

Microbiogical diagnosis of nosocomial pneumonia is particularly difficult, and sputum cultures have very low sensitivity and specificity. When microorganisms such as *E. coli, S. aureus* or Candida sp. are isolated, they may be aetiologic agents or represent oropharyngeal colonization. Thus, the approach used in differentiating them is very important.

The incidence of *L. pneumophila* varies widely from study to study, ranging from 1 to 16%. Mortality rates range from 5% in nonimmunosuppressed patients to 80% in compromised patients. Pneumonia with *L. pneumophila* and other Legionella sp. may occur in patients with a variety of defects, and its diagnosis is particularly difficult because of its weak Gram staining and the delay associated with a serological response of difficult cultures. For culturing this organism, BCYE agar is one of the media used. BCYE agar can be used for the isolation of the organism, but as respiratory specimens, especially sputum samples, include flora members, their growth does not allow easy differentiation of *L. pneumophila* colonies. Therefore, a more selective medium is recommended. BCYE-selective agar including antimicrobials for the inhibition of flora members is more useful (3,4). In our study, the identification of *L. pneumophila* was much easier when BCYE-s agar was used.

Another approach for the isolation of *L. pneumophila* is acid decontamination. If the sputum samples are acid treated and then cultured, flora members are inhibited, so isolation may be easier. In our study the results of acid treatment were similar to those obtained by culturing onto BCYE-s agar. These two techniques can be recommended and if both methods are used, accurate results may be obtained (11-13).

*L. pneumophila* is a faintly staining organism, so microscopic examination of the sputum specimens is usually insufficient. Microscopic examination is useful anly when used in combination with another identification technique.

There is increasing use of flourescent antibodies for identification of *L. pneumophila* in clinical specimens, suggesting that these methods have good sensitivity. In our study, the DFA test was applied to the sputum samples, and in 6 specimens that yielded *L. pneumophila,* positive results were obtained (85% sensitivity). Cross reaction with other organisms was not seen (100% specificity).

For serological diagnosis of Legionnaires' disease, a four-fold increase in antibody titres is required. A single high titre (≥1/128) may indicate acute disease if the prevalance is low in that population. In our study, by IFA assay the sera of 3 patients were screened for *L. pneumophila* antibodies; 1/256 titre was obtained for 2 patients, indicating acute disease. We can conclude that IFA is not sufficient for diagnosis; it must be performed in addition to another technique, especially culturing.

In conclusion, immunocompromised patients are susceptible to infections caused by a variety of infectious agents. *L. pneumophila* is one of the pathogens that cannot be isolated by routine diagnostic methods. Our study showed that it plays an important role in the pneumonia of immunocompromised patients. For microbiological diagnosis of pneumonia caused by *L. pneumophila,* culturing is the 'gold standard' especially when the specimen is acid decontaminated or inoculated onto antibiotic-supplemented media. Immunfluorescence techniques and direct microscopic examination are of diagnostic value when combined with culturing methods. Therefore, a combination of techniques must be added to the routine culturing programmes for the isolation and identification of *L. pneumophila.*

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